# VERIFICATION OF TRANSLATION

Re : Japanese Patent Application No. 2002-008435

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Dated this 31st day of August, 2007

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[Document] Specification

[Title of the Invention] Novel Phospholipase $A_2$ and Gene Thereof

[Claims]

[Claim 1] A protein selected from the following (a), (b) and (c):

(a) a protein consisting of the amino acid sequence shown in SEQ ID NO: 9;

(b) a protein consisting of an amino acid sequence in which one or more amino acids in the amino acid sequence shown in SEQ ID NO: 9 are deleted, substituted or added, wherein the protein possesses a phospholipase $A_2$ activity; and

(c) a protein encoded by a DNA capable of hybridizing with a DNA consisting of a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 8 under stringent conditions, wherein the protein possesses a phospholipase $A_2$ activity.

[Claim 2] The protein according to claim 1, which is derived from human.

[Claim 3] A gene encoding the protein as defined in claim 1.

[Claim 4] A gene consisting of a DNA of the following (a) or (b):

(a) a DNA consisting of the nucleotide sequence shown in SEQ ID NO: 8; or

(b) a DNA capable of hybridizing with a DNA consisting of a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 8 under stringent conditions, wherein the DNA encodes a protein possessing a phospholipase $A_2$ activity.

[Claim 5] The gene according to claim 4, which is derived from human.

[Claim 6] A recombinant vector comprising the gene as defined in

claim 3 or 4.

[Claim 7] The recombinant vector according to claim 6, which is an expression vector.

[Claim 8] A host cell into which the recombinant vector as defined in claim 7 is introduced.

[Claim 9] An antibody capable of recognizing the protein as defined in claim 1.

[Claim 10] A method for characterizing, identifying or screening a phospholipase $A_2$ inhibitor, comprising the steps of carrying out an enzymatic reaction in a system comprising the protein as defined in claim 1, a glycerophospholipid and a test substance, and assaying an inhibitory action of the test substance for the enzymatic activity of the protein as defined in claim 1.

[Claim 11] A method for characterizing, identifying or screening a phospholipase $A_2$ inhibitor, comprising the steps of carrying out a binding reaction in a system comprising the protein as defined in claim 1 and a test substance, and assaying whether or not the test substance has a binding activity to the protein as defined in claim 1.

[Claim 12] The method according to claim 10 or 11, wherein the method is usable for characterizing, identifying or screening a phospholipase $A_2$ inhibitor by the selectivity of the inhibitory actions for plural forms of phospholipase $A_2$.

[Claim 13] An examination method for psoriasis, characterized by assaying an expression level of the gene as defined in claim 3 or 4 for a biological sample collected from a human or non-human animal individual.

[Claim 14] The examination method according to claim 13, wherein the

assaying is carried out using a nucleic acid capable of hybridizing with a DNA consisting of the nucleotide sequence shown in SEQ ID NO: 8 under stringent conditions, or a complement thereof as a probe or primer.

[Claim 15] The examination method according to claim 13, wherein the assaying is carried out using a nucleic acid capable of hybridizing with a DNA consisting of the nucleotide sequence shown in SEQ ID NO: 4 under stringent conditions, or a complement thereof as a probe or primer.

[Claim 16] The examination method according to claim 13, wherein the assaying is carried out using an antibody capable of recognizing the protein as defined in claim 1.

[Claim 17] A method for detecting expression of the protein as defined in claim 1 in a cell or a tissue, wherein the method uses an antibody capable of recognizing the protein as defined in claim 1.

[Claim 18] A method for detecting the gene as defined in claim 3 or 4, wherein the method uses a nucleic acid capable of hybridizing with a DNA consisting of the nucleotide sequence shown in SEQ ID NO: 8 under stringent conditions, or a complement thereof as a probe or primer.

[Claim 19] A nucleic acid capable of hybridizing with a DNA consisting of the nucleotide sequence shown in SEQ ID NO: 8 under stringent conditions, or a complement thereof, wherein the nucleic acid or a complement thereof is used for controlling expression of the gene as defined in claim 3 or 4 in a cell.

[Detailed Description of the Invention]

**[0001]**

[Technical Field to Which the Invention Pertains]

The present invention relates to a novel phospholipase $A_2$ and a gene thereof associated with psoriasis.

**[0002]**

[Prior Art]

Psoriasis is one of inflammatory keratosis syndromes, and is a chronic intractable dermal disease characterized by scale. In many cases, remission and animus are repeated, and there is no decisive method for treating psoriasis to date.

**[0003]**

Psoriasis is roughly classified into five types (1) psoriasis vulgaris, (2) erythroderma psoriaticum, (3) psoriasis arthropica, (4) psoriasis guttata, and (5) pustular psoriasis, based on the pathologic characteristics. Among them, psoriasis vulgaris, of which patients are largest in number, accounts for about 80% of the patients, and pustular psoriasis is accompanied with systemic symptom, leading to death in some cases. In any type, pathology of psoriasis is accompanied with both of immunological abnormality in epidermis and dermis, and abnormality in proliferation and differentiation of an epidermic keratinocyte (Setsuya SOBA, *Jinjoseikansenno Byoin/Byotai Men-ekigakuteki Sokumen (Etiology or Pathology of Psoriasis Vulgaris, Immunological Aspect)*, *MB Derma*, **2**, 15-21, 1997; Mariko KIYOSHIMA, *Jinjoseikansenno Byoin/Hyohisaibono Zoshoku/Bunkakiko (Etiology of Psoriasis Vulgaris, Proliferation/Differentiation Mechanism of Epidermal Cells)*, *MB Derma*, **2**, 23-30, 1997).

**[0004]**

Causes of onset for psoriasis are involved in both of genetic background and exogenous factor, and the psoriasis is developed by exposing any exogenous

or endogenous factor to a person having a certain kind of genetic background.

**[0005]**

As to psoriasis, epidemiological studies have been conducted from old days, and its genetic forms have been known to be multifactorial. From the parent-child incidence rate of psoriasis, it is deduced that psoriasis takes the form of a prepotency. Also, it is considered from the congruity rate between the twins that the number of genes involved in the incidence is not so large (Atsuyuki IGARASHI, *Jinjoseikansenno Ekigaku (Epidemiology of Psoriasis Vulgaris), MB Derma*, **2**, 1-5, 1997; Akira OZAWA, *Jinjoseikansenno Iden (Heredity of Psoriasis Vulgaris), MB Derma*, **2**, 7-13, 1997).

**[0006]**

PSOR1 (Psoriasis susceptibility) gene existing in HLA antigen gene region of chromosome 6 is a psoriasis-sensitive gene which was happened to be found by typing of the HLA during the kidney transplantation. As genes involved in the onset of psoriasis, in addition to this PSOR1 gene, several kinds of genes localized in chromosome 16, chromosome 17, and chromosome 20, have been reported to date (Muneo OKIDO, *Kansento Sonokanjusei Idenshi (Psoriasis and Susceptible Gene Thereof), The Japanese Journal of Dermatology*, **105**, 1505-1510, 1995).

However, there are many points in the mechanisms of onset of psoriasis which have not yet been clarified, and the development of a therapeutic method or the like is difficult. In order to connect to the development of a new diagnostic or therapeutic method, advancements in research and analysis of a gene involved in the onset of psoriasis (psoriasis-associated gene) has been desired.

[0007]

On the other hand, the phospholipase $A_2$ has been known as an enzyme heavily involved in inflammatory diseases such as psoriasis. The phospholipase $A_2$ is an enzyme which catalyzes a reaction in which an ester bond at 2-position of a glycerophospholipid is hydrolyzed to generate equimolar amounts of a free fatty acid and a lysophospholipid. Arachidonic acid released from glycerophospholipid constituting a biomembrane by action of phospholipase $A_2$ is converted into leukotrienes by lipoxygenase or the like, or converted into prostaglandins with cyclooxygenase or the like, or further converted into thromboxane $A_2$ or the like by action of thromboxane synthase on the prostaglandins. Since it has been revealed that these eicosanoids (leukotrienes, prostaglandins, and thromboxanes) are closely involved with various inflammations, allergic reactions, ischemic diseases, and the like, the phospholipase $A_2$ plays a key role as an enzyme which causes a primary reaction that leads to production of inflammatory mediators downstream of arachidonic acid.

[0008]

It has been known that the phospholipase $A_2$ has at least 17 kinds of molecular species. Those molecular species are classified into four subclasses (secretory phospholipase $A_2$, cytoplasmic phospholipase $A_2$, $Ca^{2+}$-independent phospholipase $A_2$, and platelet activating factor acetylhydrolase), based on similarities in structure and characteristics.

[0009]

Among them, as cytoplasmic phospholipase $A_2$ ($cPLA_2$), which is high-molecular weight phospholipase $A_2$ localized in a cytoplasm, three molecular

species ($cPLA_2\alpha$, $cPLA_2\beta$, $cPLA_2\gamma$) have been so far identified. $cPLA_2\gamma$ exhibits activity in a $Ca^{2+}$ concentration-independent manner. On the other hand, $cPLA_2\alpha$ and $cPLA_2\beta$ each has a phospholipid-binding region at N-terminal side of a protein molecule, and exhibits its activity by binding to a phospholipid membrane in a $Ca^{2+}$ concentration-dependent manner. However, since the amount of $Ca^{2+}$ required for exhibiting the activity is on the order of μM, it is considered that these also exhibit their activities in cytoplasm.

**[0010]**

The phospholipase $A_2$ is an important target molecule in the development and research of therapeutic drugs for inflammatory diseases and the like, and studies of inhibitors thereof have been advanced intensively. There are some cases where inhibitory action for the phospholipase $A_2$ has been found in known medicaments. In addition, it has been found that a specific phospholipase $A_2$ inhibitor can serve as a useful therapeutic drug.

**[0011]**

In order to develop an excellent medicament having high therapeutic effects and little side effects, it has been desired to screen an inhibitor having a high selectivity on a particular type of the phospholipase $A_2$ to be targeted.

**[0012]**

Further, it has been also desired to find a novel type of the phospholipase $A_2$, which is a different molecular species from those of conventional ones, for the studies of the mechanism of onset of inflammatory diseases, and from the possibility to be used as a target molecule of a novel therapeutic drug.

**[0013]**

[Problems to Be Solved by the Invention]

An object of the present invention is to provide a novel type of a phospholipase $A_2$ and a gene thereof, associated with psoriasis. In addition, there are provided a novel method for characterizing, identifying or screening a phospholipase $A_2$ inhibitor, and a novel method for diagnosis and examination for psoriasis. Also, objects of the present invention other than the objects as described above will be apparent from the following description.

**[0014]**

[Means to Solve the Problems]

Using the body map method developed by Okubo et al. (*Methods in Molecular Genetics*, **5**, 17-33, 1994), the present inventors have found a psoriasis-associated gene (GS21015 gene), of which expression is specifically increased in epidermis of a patient with psoriasis, by intensively comparing and studying genes expressed in epidermis of a patient with psoriasis with those of a normal individual. Further, the present inventors have found that a protein encoded by the gene is a novel phospholipase $A_2$, and succeeded in expression of the phospholipase $A_2$ in a cell by genetic recombination technique. The present invention has been completed thereby.

**[0015]**

Concretely, the present invention is a protein selected from the following (a), (b) and (c):

(a) a protein consisting of the amino acid sequence shown in SEQ ID NO: 9;

(b) a protein consisting of an amino acid sequence in which one or more amino acids in the amino acid sequence shown in SEQ ID NO: 9 are deleted, substituted or added, wherein the protein possesses a phospholipase $A_2$ activity; and

(c) a protein encoded by a DNA capable of hybridizing with a DNA consisting of a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 8 under stringent conditions, wherein the protein possesses a phospholipase $A_2$ activity.

**[0016]**

Also, the present invention is a gene encoding the above-mentioned protein, and a recombinant vector and a host cell, each containing the gene. In addition, the present invention is an antibody capable of recognizing the above-mentioned protein.

**[0017]**

Further, the present invention is a method for characterizing, identifying or screening a phospholipase $A_2$ inhibitor using the above-mentioned protein. In addition, the present invention is an examination method for psoriasis, characterized by assaying an expression level of a gene encoding the above-mentioned protein.

**[0018]**

SEQ ID NO: 8 in the Sequence Listing set forth below shows a nucleotide sequence of a human cDNA containing a full length translational region of a psoriasis-associated gene (GS21015 gene) found by the inventors, and SEQ ID NO: 9 shows an amino acid sequence of a human protein (GS21015) encoded by the above-mentioned full length cDNA.

**[0019]**

Searches for homology for the nucleotide sequence shown in SEQ ID NO: 8 and the amino acid sequence shown in SEQ ID NO: 9 are conducted on the bases of the known DNA database (GenBank and EMBL) and

the protein database (NBRF and SWISS-PROT). As a result, those that are considered to be derived from the same molecular species have not been found.

**[0020]**

The novel phospholipase $A_2$ and a gene thereof of the present invention are useful as a novel target molecule in research and development of a therapeutic agent for a disease, especially a therapeutic agent for an inflammatory disease. Inter alia, expression of the phospholipase $A_2$ and the gene thereof is increased in a psoriatic tissue as compared to a normal tissue in a skin tissue, and the phospholipase $A_2$ and the gene thereof are specifically expressed thereto. Therefore, the phospholipase $A_2$ and the gene thereof are useful in research and development of a therapeutic agent for an inflammatory dermal disease such as psoriasis. In addition, the phospholipase $A_2$ and the gene thereof are also useful in studies of onset mechanism of an inflammatory disease.

**[0021]**

Further, the method for characterizing, identifying and screening an inhibitor utilizing a novel phospholipase $A_2$ of the present invention is useful for the development of an inhibitor having a high selectivity and an excellent medicament having a high therapeutic effect and a little side effect.

**[0022]**

In addition, since expression of the novel phospholipase $A_2$ and the gene thereof of the present invention is increased in a psoriatic tissue as compared to a normal tissue in a skin tissue, the examination method of the present invention characterized by assaying the expression levels of these phospholipase $A_2$ and the gene thereof is useful in diagnosis and examination of the pathological condition of psoriasis.

[0023]

[Modes for Carrying out the Invention]

The protein of the present invention includes, for instance, a protein consisting of the amino acid sequence shown in SEQ ID NO: 9. In addition, the protein includes a protein consisting of an amino acid sequence in which one or more amino acids are deleted, substituted or added in the amino acid sequence shown in SEQ ID NO: 9. Deletion, substitution, or addition of the amino acids may be to an extent that the functions as the phospholipase $A_2$ (biological activity), more specifically an activity of hydrolyzing an ester bond of a glycerophospholipid at 2-position (phospholipase $A_2$ activity) are not lost, and is usually 1 to about 200 in number, preferably 1 to about 160 in number, more preferably 1 to about 120 in number, further preferably 1 to about 80 in number, still further preferably 1 to about 40 in number.

[0024]

More particularly, besides the protein having the amino acid sequence shown in SEQ ID NO: 9, the protein of the present invention includes a protein having one or more conservative amino acid substitutions as compared to the protein consisting of the amino acid sequence shown in SEQ ID NO: 9.

[0025]

The protein as described above encompasses variant proteins found in nature as well as artificially modified variant proteins, proteins derived from heterogeneous organisms, and the like.

[0026]

In other words, the protein as described above includes conservative substitution variants and naturally occurring allelic variants of the protein

consisting of the amino acid sequence shown in SEQ ID NO: 9.

**[0027]**

The protein as described above has a homology on an amino acid level of usually about 75% or more, preferably about 80% or more, more preferably about 85% or more, further preferably about 90% or more, still further preferably about 95% or more, with the amino acid sequence shown in SEQ ID NO: 9.

**[0028]**

In addition, in order not to lose its function as a phospholipase $A_2$ of the protein as described above, it is desirable that an amino acid sequence is more highly conserved in a region controlling the phospholipase $A_2$ activity, i.e., a catalytic region, than in other regions. The catalytic region of the protein consisting of the amino acid sequence shown in SEQ ID NO: 9 includes, for instance, regions corresponding to 275th to 525th amino acid residues (catalytic region A) and 613th to 798th amino acid residues (catalytic region B) in the amino acid sequence shown in SEQ ID NO: 9. Deletion, substitution or addition of the amino acids in each of the catalytic regions is usually 1 to about 20 in number, preferably 1 to about 10 in number, more preferably 1 to about 5 in number, respectively. Each of the catalytic regions of the protein described above has a homology on an amino acid level of usually about 90% or more, preferably about 95% or more, more preferably about 97% or more, respectively, with each of the catalytic regions existing in the amino acid sequence shown in SEQ ID NO: 9. On the other hand, deletion, substitution or addition of the amino acids in the non-catalytic region is usually 1 to about 160 in number, preferably 1 to about 140 in number, more preferably 1 to about 110 in number, further preferably 1 to about 80 in number, still further preferably 1 to about 40

in number.

**[0029]**

The gene of the present invention includes a gene consisting of a DNA consisting of the nucleotide sequence shown in SEQ ID NO: 8. In addition, the gene includes a gene consisting of a DNA capable of hybridizing with a DNA consisting of a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 8 under stringent conditions. The hybridizing DNA as described above may be any of those encoding a protein having a phospholipase $A_2$ activity.

**[0030]**

The gene as described above has a homology of usually 70% or more, preferably about 80% or more, more preferably about 85% or more, further preferably 90% or more, still further preferably 95% or more, with the nucleotide sequence shown in SEQ ID NO: 8. The gene as described above encompasses variant genes found in nature, artificially modified variant genes, homologous genes derived from heterogeneous organisms (orthologs), and the like.

**[0031]**

In the present invention, the hybridization under stringent conditions can be usually carried out by hybridizing under a temperature condition of 50° to 60°C for about 16 hours in 6 × SSC or in a hybridization solution having a salt concentration equivalent thereto, pre-washing in 6 × SSC or in a solution having a salt concentration equivalent thereto if needed, and thereafter washing in 1 × SSC or in a solution having a salt concentration equivalent thereto. In addition, under the conditions of even higher stringency (under high-stringent conditions), the hybridization can be carried out by washing in 0.1 × SSC or in a

solution having a salt concentration equivalent thereto in the above-mentioned hybridization.

**[0032]**

The gene of the present invention can be isolated and obtained screening a tissue and a cell of a mammal as a gene source. The mammal includes a non-human animal such as dog, cattle, horse, goat, sheep, monkey, pig, rabbit, rat and mouse, as well as human. Among them, for the utilization in research and development of therapeutic agents for human, it is desirable to use those derived from human.

**[0033]**

The gene of the present invention can be obtained by utilizing the sequence information (SEQ ID NO: 8 in the Sequence Listing set forth below) disclosed herein. For instance, primers and probes are designed on the basis of the information of the disclosed nucleotide sequences, so that the nucleic acid can be selected and obtained from a DNA library by appropriately combining PCR (polymerase chain reaction) method, colony hybridization method and plaque hybridization method using these primers and probes.

**[0034]**

For instance, a cDNA is synthesized from mRNA prepared from a cell or a tissue of a mammal, and a cDNA fragment is obtained by PCR method using the resulting cDNA as a template. A cDNA library is screened by colony hybridization method or plaque hybridization method using the resulting cDNA fragment as a probe, whereby a full length cDNA can be obtained. Also, a genomic gene can be isolated by screening a genomic DNA library. In addition, the homologous gene (ortholog) derived from a heterogeneous organism can be

isolated by screening a DNA library of other mammal.

**[0035]**

The DNA library such as a cDNA library and a genomic DNA library can be prepared in accordance with the method described in "*Molecular Cloning*" (authored by Sambrook, J., Fritsch, E. F. and Maniatis, T., published on 1989 by Cold Spring Harbor Laboratory Press). Alternatively, when there is a commercially available library, this library may be used.

**[0036]**

By sequencing the resulting cDNA, a translational region encoding a protein which is the gene product can be determined, so that an amino acid sequence of this protein can be obtained.

**[0037]**

The protein of the present invention can be produced by overexpression according to a conventional gene recombination technique. Alternatively, the protein can also be expressed and produced in the form of a fusion protein with other protein or peptide.

**[0038]**

The cell in which the protein of the present invention is allowed to be overexpressed can be obtained, for instance, as follows. First, a DNA encoding the protein of the present invention is inserted into a vector in the form that is ligated at downstream of an appropriate promoter, to construct an expression vector. Next, the resulting expression vector is introduced into a host cell.

**[0039]**

The expression system (host-vector system) includes, for instance, expression systems of bacterium, yeast, insect cell and mammal cell, and the like.

Among them, in order to obtain a protein having a well conserved function, it is preferable to use as a host a mammal cell (monkey COS-7 cell, Chinese hamster CHO cell, human HeLa cell and the like) and an insect cell (*Spodoptera frugiperda* SF9, SF21 and the like).

**[0040]**

As the promoter for expressing the protein of the present invention, SV40 promoter, LTR promoter, elongation 1α promoter or the like can be used in the system of a mammal cell, and polyhedrin promoter or the like can be used in the case of the system of an insect cell.

**[0041]**

As the vector, a retroviral vector, a papilloma virus vector, a vaccinia virus vector, an SV40 vector or the like can be used in the case of the system of a mammal cell, and a baculovirus vector or the like can be used in the case of the system of an insect cell.

**[0042]**

As the DNA encoding the protein of the present invention, there can be used, but not being limited thereto, a cDNA corresponding to a naturally occurring mRNA (for instance, one consisting of the nucleotide sequence shown in SEQ ID NO: 8). A DNA corresponding to an amino acid sequence of the desired protein can be designed and used. In this case, as a codon encoding one amino acid, 1 to 6 kinds of codons each have been known. The codon used may be arbitrarily selected. For instance, by taking into consideration the codon usage frequency by a host utilized in expression, a sequence showing an even higher expression efficiency can be designed. The DNA having the designed nucleotide sequence can be obtained by chemical synthesis of a DNA, binding

with a fragment of the above-mentioned cDNA, partial modification of the nucleotide sequence and the like. The artificial modification of a part of a nucleotide sequence, and introduction of mutation can be carried out by PCR method utilizing primers each consisting of a synthetic oligonucleotide encoding the desired modification, site specific mutagenesis (Mark et al., *Proceedings of National Academy of Sciences*, **81**, 5662-5666, 1984), and the like.

**[0043]**

The protein of the present invention can be separated and purified by appropriately combining the known purification methods (salting-out with an inorganic salt, fractionation precipitation with an organic solvent, ion-exchanging resin column chromatography, affinity column chromatography, gel filtration and the like) from a culture of a cell into which an expression vector is introduced and the like.

**[0044]**

The function or activity of the protein in a cell can be increased (enhanced) by overexpression of the protein of the present invention.

**[0045]**

The nucleic acid capable of hybridizing with the DNA constituting the gene of the present invention (DNA or RNA: oligonucleotide or polynucleotide) under stringent conditions, or a complement thereof (a nucleic acid having a complementary sequence thereof) can be used as a probe or a primer for detecting the gene of the present invention. Alternatively, there can be used as, for instance, an antisense oligonucleotide, a ribozyme or decoy, in order to change (for instance, suppress) expression of the gene. As the nucleic acid or a complement thereof as described above, there can be used, for instance, a

nucleotide having a partial sequence of 14 or more consecutive nucleotides of the nucleic acid consisting of the nucleotide sequence shown in SEQ ID NO: 8, or a complementary sequence thereof.

**[0046]**

The antibody capable of recognizing the protein of the present invention can be obtained by using as an antigen the protein of the present invention or a protein or peptide having immunological equivalence thereto (synthetic peptide having a fragment of the protein or a partial sequence thereof and the like). Having immunological equivalence as used herein means, for instance, the generation of cross-reaction with an antibody against the protein of the present invention.

**[0047]**

The polyclonal antibody can be produced by a usual method comprising inoculating an antigen to a host animal (for instance, rat, rabbit or the like), and then collecting immune serum. The monoclonal antibody can be produced by a technique of a usual hybridoma method or the like. Alternatively, a humanized monoclonal antibody or the like can be produced by modifying a gene of the monoclonal antibody.

**[0048]**

Expression of the protein of the present invention in a cell, a tissue or the like can be detected by a usual immunochemical method (immunochemical assay method or the like) using the antibody obtained as mentioned above. Alternatively, the purification of the protein of the present invention can be carried out by affinity chromatography with an antibody. In addition, the function or activity of the protein of the present invention can be changed (for

instance, suppressed) by using a neutralizing antibody.

**[0049]**

The presence of a phospholipase $A_2$ activity of the protein of the present invention can be confirmed, for instance, by a generally known, usual method for measuring a phospholipase $A_2$ activity (Underwood et al., *The Journal of Biological Chemistry*, **273**, 21926-21932, 1998).

**[0050]**

The protein of the present invention can be used for characterizing, identifying or screening a phospholipase $A_2$ inhibitor.

**[0051]**

For instance, an enzymatic reaction is carried out in a system containing the above protein, a glycerophospholipid and a test substance (preferably a low-molecular compound or the like), to assay an inhibitory action of the test substance on an enzyme activity (an activity of hydrolyzing an ester bond of a glycerophospholipid at 2-position).

**[0052]**

Alternatively, a binding reaction is carried out in a system containing the above protein and a test substance (preferably a low-molecular compound or the like), to assay whether or not the test substance has a binding activity to the above protein. There is a high possibility that a test substance (ligand) having a binding activity serves as an inhibitor.

**[0053]**

Further, the selectivity of the inhibitory action (or the binding activity) can be judged by evaluating the inhibitory action (or the binding activity) on the above protein for a test substance (preferably a low-molecular compound or the

like), and comparing with an inhibitory action (or binding activity) on other types of phospholipase $A_2$. By the judgment, an inhibitor having a relatively high action on a particular type of the phospholipase $A_2$ (selective inhibitor) can be screened. In addition, the inhibitor can be identified and characterized.

**[0054]**

Also, the gene encoding the protein of the present invention, that is, the gene of the present invention is a gene of which expression is increased in a psoriatic tissue as compared to a normal tissue. Therefore, the pathological conditions of psoriasis, and the like can be diagnosed or examined by assaying an expression level of the gene of the present invention in a biological sample. The examination method of the present invention described above can be utilized in, for example, judgment of whether or not an individual suffers from psoriasis, judgment on the characteristics of pathological conditions of psoriasis, judgment on severity of psoriasis, judgment on the effect of a therapeutic agent or a method of treating on psoriasis, or the like. Inter alia, the examination method of the present invention is preferably applied to psoriasis vulgaris. In addition, the examination method of the present invention can be applied to a psoriatic model of a mammal such as monkey, dog, rat and mouse, besides the application to human psoriasis.

**[0055]**

This examination method is carried out by collecting and preparing a biological sample from a human or non-human animal individual (an individual suffering from psoriasis, an individual being suspected of suffering from psoriasis and the like), and thereafter assaying an expression level of the gene of the present invention using this biological sample.

[0056]

The biological sample includes a cell, a tissue, and the like, derived from an animal (human or non-human) individual. The tissue includes a skin tissue, and especially a skin tissue at a psoriatic lesion site such as desquamatory is preferable. The cell includes an epidermal cell existing in the skin tissue or the like.

[0057]

The assay for the expression level of the gene of the present invention in a biological sample can be carried out, for instance, by extracting mRNA from a biological sample to detect or assay mRNA derived from the gene of the present invention existing in this mRNA (or corresponding cDNA). Alternatively, the protein of the present invention which is a gene product may be detected or assayed.

[0058]

In the detection or the assay of mRNA (or corresponding cDNA) from the gene of the present invention, RT-PCR (reverse transcriptase-polymerase chain reaction) method ("*PCR Protocols*" Innis MA, Gelfad DH, Sninsky JJ and White TJ eds., Academic Press, San Diego, 1990); iAFLP method utilizing RT-PCR method (Kawamoto et al., *Genome Research*, **9**, 1305-1312, 1999), DNA microarray method or DNA microchip method (Schena et al., *Science*, **270**, 467-470, 1995; Fodor et al., *Science*, **251**, 767-773, 1991), usual Northern blotting method and the like can be utilized.

[0059]

Among them, the RT-PCR method is preferable from the viewpoints of sensitivity and operability. Also, the iAFLP method in which the RT-PCR

method is applied is a method which is made high-throughput more efficiently than the RT-PCR method, and is preferable for assaying a large number of samples.

**[0060]**

For instance, when the RT-PCR method is utilized, appropriate primers for amplifying a fragment containing a region having a specific nucleotide sequence in mRNA (or a corresponding cDNA) from the gene of the present invention are designed and synthesized. Using these primers, PCR is carried out with a cDNA synthesized from mRNA in a biological sample as a template. The resulting PCR product is separated by electrophoresis or the like, if needed, and a fragment thereof may be detected to assay the existing amount thereof.

**[0061]**

In the detection and the assay of mRNA (or a corresponding cDNA), usually, a nucleic acid (oligonucleotide or the like) capable of hybridizing with a region having a specific nucleotide sequence among mRNA (or corresponding cDNA) from the gene of the present invention, or a complement thereof is designed and synthesized, and this is used as a probe or a primer.

**[0062]**

The region having a specific nucleotide sequence is not particularly limited. Since a region on a 3'-terminal side of mRNA (or a corresponding cDNA) has a high sequence specificity in the individual genes, it is preferable to employ a probe or a primer corresponding to this region from the viewpoint that the gene expression level is appropriately reflected in the detection or assay. The region on a 3'-terminal side in a gene of the present invention includes, for instance, a region from polyA to *Mbo*I recognizing site appearing first upstream

therefrom in mRNA of the GS21015 gene, concretely, a region having the nucleotide sequence shown in SEQ ID NO: 4.

**[0063]**

When expression of the gene of the present invention is detected or assayed by detecting the protein of the present invention, which is the gene product, for instance, an antibody which is capable of specifically recognizing the protein of the present invention is produced by the above-mentioned method, and a method of detection by the usual immunochemical method can be used utilizing this antibody.

**[0064]**

The present invention will be described hereinafter more specifically by means of Examples, without intending to limit the present invention to these Examples.

**[0065]**

In the following Examples, unless specified otherwise, each of the procedures was carried out in accordance with the method described in "*Molecular Cloning*" (authored by Sambrook, J., Fritsch, E. F. and Maniatis, T., published on 1989 by Cold Spring Harbor Laboratory Press), or by using an instruction manual of a commercially available product when a commercially available reagent or kit is used.

**[0066]**

[Examples]

Example 1 Profiling of Psoriasis-Associated Gene by Body Map Method

Random screening of a psoriasis-associated gene was carried out by Body Map method, in accordance with the method described in the literature of Okubo

et al. (*Methods in Molecular Genetics*, **5**, 17-33, 1994), as follows.

**[0067]**

(1) Preparation of Vector Primer

A vector plasmid pUC119 (manufactured by Takara Shuzo Co., Ltd.) was digested with restriction enzyme *Pst*I, and thereafter the digested product was reacted with [$^3$H]dTTP and terminal transferase to add oligo dT (about 30 to 35 in number) to the terminal. There was used a vector plasmid pUC119 which had been prepared by replication using *dam*+ bacterium (strain name: DH5α; Toyobo Co., Ltd.) as a host so as not to be digested with restriction enzyme *Mbo*I. The state for addition of oligo dT was monitored by incorporation of RI radioactivity. Subsequently, this resulting vector was digested with *Hin*cII. Thereafter, the digested product was extracted with phenol-chloroform and precipitated by ethanol for several times. Further, a longer vector fragment was purified and obtained using oligo(dA) cellulose column. This vector fragment was used as a primer (vector primer).

**[0068]**

(2) Preparation of 3'-Oriented cDNA Library and Determination of Nucleotide Sequence of Each Clone

Each of one case of a skin tissue (desquamatory skin tissue of lesion site) collected from a psoriatic patient and one case of a skin tissue collected from a normal individual was physically disrupted in Cool Mill (manufactured by Toyobo Co., Ltd.). Thereafter, a total RNA was prepared from these samples using RNA preparation kit (RNeasy kit, manufactured by QIAGEN).

**[0069]**

With the thus obtained total RNA (about 1 μg each) (derived from a

psoriasis patient or a normal individual) as a template, a single-stranded cDNA was synthesized from a 3' side of the template, using 50 ng of the vector primer prepared in the above (1) as a primer, and reverse transcriptase (trade name: Superscript II; manufactured by BRL) of MMLV (Moloney murine leukemia virus). Thereafter, a double-stranded cDNA was synthesized using polymerase, ligase, and the like. Further, a cDNA was purified and obtained using Glass Milk (manufactured by BIO 101) (or by extraction with phenol-chloroform and ethanol precipitation).

**[0070]**

The resulting double-stranded cDNA (the one added to vector) was digested with restriction enzyme *Mbo*I (recognizing GATC sequence) and *Bam*HI. The digested product was then ligated with *Mbo*I adapter, and thereafter a fragment containing a vector was circularized. The resulting product was introduced into *Escherichia coli* (strain name: DH5α; Toyobo Co., Ltd.) to give a transformant.

**[0071]**

A colony of the resulting transformant (about 10000 each for the clones derived from tissues of normal individuals and from psoriatic patients) was picked up on a 96-well plate using an automatic bacterium-picking up device. The cells were cultured on the 96-well plate, and thereafter heated in an autoclave (90°C for 20 minutes) to lyse the cells. The cell lysate was filtered with Millipore filter to obtain supernatant. PCR was carried out using the resulting supernatant as a template to amplify an insert in a vector plasmid. PCR was carried out for 27 cycles under the conditions that one cycle comprises 93°C for 30 seconds, 50°C for 60 seconds and 72°C for 2 minutes, followed by one

cycle under the conditions of 72°C for 5 minutes as a final cycle, and the reaction was terminated. In addition, the primers for PCR were designed on the basis of a nucleotide sequence of a vector part nearby the insert. As a sense primer, there was used oligonucleotide (FW(-40)) having the nucleotide sequence shown in SEQ ID NO: 1, and as an antisense primer, there was used oligonucleotide (RV(-14)) having the nucleotide sequence shown in SEQ ID NO: 2, in the Sequence Listing set forth below.

**[0072]**

Next, the PCR product obtained above was subjected to a sequencing reaction in accordance with the dideoxy method, and thereafter a nucleotide sequence was determined using an automatic DNA sequencer (373A, manufactured by Applied Biosystems). As a primer for a sequencing reaction, there was used an oligonucleotide of the nucleotide sequence shown in SEQ ID NO: 3 from a vector (vector primer).

**[0073]**

Thus, 10000 each of cDNA clones from normal and psoriatic skin tissues were subjected to nucleotide sequencing, and clones for which nucleotide sequences could not be decoded and clones from mitochondria were removed. As a result, the nucleotide sequence information of about 4300 clones from normal tissues and about 3700 clones from psoriatic tissues was obtained. As the kinds of genes expressed in human skin tissues (psoriatic and normal), the genetic information of a total of about 3000 kinds was obtained.

**[0074]**

Among the genetic information obtained above, the information on the cDNA sequence obtained for clone GS21015 (i.e. a nucleotide sequence from

polyA at 3'-terminal of cDNA to *Mbo*I site appearing first) is as shown in SEQ ID NO: 4 in the Sequence Listing set forth below.

**[0075]**

Example 2: Analysis of Expression Profile by iAFLP Method

As to the clones obtained in Example 1 mentioned above, a difference in expression profile between the psoriatic tissues and the normal tissues was analyzed by iAFLP (introduced amplified fragment length polymorphism) method utilizing RT-PCR (reverse transcriptase-polymerase chain reaction) in accordance with the method described in the literature of Kawamoto et al. (*Genome Research*, **9**, 1305-1312, 1999).

**[0076]**

First, a total RNA was prepared from each of 10 cases of skin tissues collected from psoriatic patients (desquamatory skin tissue of lesion site) (referred to as P01, P02, P03, ..., P10), and 7 cases of skin tissues collected from normal individuals (referred to as N01, N02, N03, ..., N07).

**[0077]**

A 3'-side double-stranded cDNA (the one added to the vector) was obtained by carrying out the treatment in the same manner as that of the above (2) of Example 1 using the resulting RNA as a template and the vector primer prepared in the above (1) of Example 1 for PCR. The thus obtained 3'-side double-stranded cDNA (added to the vector) was used hereinbelow as a cDNA pool derived from each tissue.

**[0078]**

A total amount of each of the cDNA pools obtained above was digested with a restriction enzyme *Mbo*I (recognizing GATC sequence), and thereafter an

amplification adapter was ligated to each of the digested products. Five kinds of adapters which are different in a length by 3 bases each (LP40, LP43, LP46, LP49, and LP52) were used as the amplification adapters, in the following combinations so that PCR products can be distinguished even when subjected to simultaneous treatment.

| | |
|---|---|
| P01, P06, N01, N06 | → ligated with LP40 (40 bases in length) |
| P02, P07, N02, N07 | →ligated with LP43 (43 bases in length) |
| P03, P08, N03 | →ligated with LP46 (46 bases in length) |
| P04, P09, N04 | →ligated with LP49 (49 bases in length) |
| P05, P10, N05 | →ligated with LP52 (52 bases in length) |

In addition, as a standard (reference), a mixture of 30 kinds of commercially available cDNA libraries from human organs was used in place of each of the cDNA pools, and a 6th kind of the amplification adapter (LP55; 55 bases in length) was ligated thereto.

**[0079]**

PCR was carried out with the cDNA ligated with amplification adapters as mentioned above as a template. As the sense primer for PCR, there was used a fluorescent-labeled oligonucleotide (F-T7(25) primer, manufactured by PE Biosystems) (2 pmole) having a common nucleotide sequence to the 6 kinds of amplification adapters. As the antisense primer, there was used an oligonucleotide (2 pmole) designed and synthesized on the basis of a sequence which is unique to a gene (clone) of which expression level is to be detected (i.e., a specific sequence in the nucleotide sequence from 3'-terminal polyA of cDNA

to *Mbo*I site appearing first). Concretely, as an antisense primer for detecting gene expression of clone GS21015, an oligonucleotide having the nucleotide sequence shown in SEQ ID NO: 5 was used.

**[0080]**

Equivolumes of 3 to 5 kinds of templates ligated with different kinds of amplification adapters, and a standard template in one reaction solution (10 μl) as follows were mixed, and PCR was carried out.

Reaction solution 1 = N01, N02, P03, P04, P05, and standard
Reaction solution 2 = N03, N04, P01, P02, P06, and standard
Reaction solution 3 = N05, N06, P07, P08, and standard
Reaction solution 4 = N07, P09, P10, and standard

The reaction for PCR was carried out for 35 cycles under the conditions that one cycle comprises 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, followed by one cycle under the conditions of 72°C for 30 seconds as a final cycle, and the reaction was terminated.

**[0081]**

The resulting PCR product was electrophoresed using ABI automatic sequencer (manufactured by ABI) to detect and quantify the fluorescent light.

**[0082]**

As to the detection value ascribed to the cDNA pool of each tissue sample, a relative value was calculated to a detected value ascribed to the standard cDNA, and this value was defined as a relative value of an expression level of a gene (transcript) in each tissue sample.

[0083]

As mentioned above, the gene expression profile of the clone GS21015 was analyzed. The results are as shown in Figure 1.

[0084]

The gene corresponding to the clone GS21015 (GS21015 gene) showed a higher expression level in a skin tissue from a psoriatic patient as compared to those in a skin tissue from a normal individual. Since the GS21015 gene shows an expression profile specific to a psoriatic patient tissue, it was found that the gene can be used for diagnosing or examining pathological conditions of psoriasis.

[0085]

Example 3 Cloning of GS21015 Gene (Full Length cDNA)

As to the clone GS21015 of which a gene expression profile was confirmed in Example 2 mentioned above, a cDNA containing a full length translation region was obtained as follows.

[0086]

The known DNA database (GenBank and EMBL) were searched using BLAST program on the basis of the sequence information obtained in Example 1 (cDNA nucleotide sequence from 3'-terminal of polyA to *Mbo*I site appearing first: SEQ ID NO: 4). As a result, the sequence was hit with a genome draft sequence. Exon was extracted from this draft sequence using an exon extraction software (GeneScan), and translated into an amino acid sequence, and then searched for protein database (NBRF and SWISS-PROT) by using the amino acid sequence. As a result, the exon showed a high homology with a cytoplasmic phospholipase $A_2$ ($cPLA_2$).

[0087]

PCR primers (SEQ ID NOs: 6 and 7) were designed on the basis of this hypothetical exon information. According to PCR using these primers, a 3'-terminal cDNA fragment (1246 bp) of the GS21015 gene was obtained from a cDNA from a psoriatic tissue.

[0088]

Further, a cDNA library from a psoriatic tissue was screened using this cDNA fragment as a probe. As the cDNA library, there was used one prepared by inserting into λZap vector a cDNA synthesized with mRNA prepared from a skin tissue (scaly skin tissue of lesion site) of a psoriatic patient as a template. As a result of screening, a full length cDNA containing a full length translation region of the GS21015 gene was obtained.

[0089]

The resulting full length cDNA was sequenced and analyzed. The nucleotide sequence (3587 bp) of the full length cDNA was shown in SEQ ID NO: 8, and the amino acid sequence (818 amino acid residues) of a protein encoded thereby was shown in SEQ ID NO: 9. The molecular weight of a protein deduced from the amino acid sequence was about 92 kDa.

[0090]

In addition, as to the nucleotide sequence shown in SEQ ID NO: 8, all the sequences contained in the known DNA database (GenBank and EMBL) were subjected to homology searching using FASTA and BLAST programs. Further, as to the amino acid sequence shown in SEQ ID NO: 9, all the sequences contained in the protein database (NBRF and SWISS-PROT) were subjected to homology searching using FASTA and BLAST programs.

**[0091]**

As a result of homology searching and analysis, it was found that the amino acid sequence shown in SEQ ID NO: 9 has partial homology with an amino acid sequence of cytoplasmic phospholipase $A_2$ ($cPLA_2$).

**[0092]**

The amino acid sequence of GS21015 shown in SEQ ID NO: 9, and amino acid sequences of $cPLA_2\alpha$, $cPLA_2\beta$ and $cPLA_2\gamma$, which have been conventionally known as molecular species of $cPLA_2$, were compared. The results are shown in Figure 2.

**[0093]**

From the similarity of the amino acid sequences, it was deduced that a catalytic region of GS21015 is a region corresponding to 275th to 525th amino acid residues (catalytic region A) and 613th to 798th amino acid residues (catalytic region B). In addition, it was deduced that GS21015 has a phospholipid binding region at N-terminal side (region corresponding to 24th to 145th amino acid residues), and is capable of binding to a phospholipid membrane in a $Ca^{2+}$ concentration-dependent manner, in the same manner as in $cPLA_2\alpha$ and $cPLA_2\beta$.

**[0094]**

When the amino acid sequence of GS21015 was compared with the amino acid sequences of $cPLA_2\alpha$, $cPLA_2\beta$ and $cPLA_2\gamma$, in the catalytic region A, there were shown homologies of 31.3% for $cPLA_2\alpha$, 66.0% for $cPLA_2\beta$ and 31.7% for $cPLA_2\gamma$, and in the catalytic region B, there were shown homologies of 28.9% for $cPLA_2\alpha$, 51.4% for $cPLA_2\beta$ and 36.4% for $cPLA_2\gamma$. In the phospholipid binding region, there were shown homologies of 31.3% for $cPLA_2\alpha$, and 45.9%

for $cPLA_2\beta$.

**[0095]**

It was deduced from the results of the comparison and analysis of these amino acid sequences that GS21015 is a member of the family of $cPLA_2$, and inter alia, a molecular species which is closest to $cPLA_2\beta$.

**[0096]**

In addition, it was found from the information of the genome draft sequence that the GS21015 gene is localized on chromosome 15, the same as in $cPLA_2\beta$.

**[0097]**

Example 4 Over expression of GS21015

The GS21015 gene (full length cDNA) obtained in Example 3 mentioned above was amplified by PCR. In the amplification, as PCR primers there were used primers which were designed so that *Eco*RI recognition site was added to each of 5'-terminal and 3'-terminal of the cDNA fragment.

**[0098]**

Next, the resulting PCR product (cDNA fragment containing full length translation region of GS21015) was inserted into *Eco*RI recognition site of vector plasmid pcDNA4 HisMax (manufactured by Invitrogen), thereby constructing an expression vector plasmid for GS21015. This expression vector plasmid is constructed so as to express a protein in which histidine (His) tag was added to the N-terminal of GS21015.

**[0099]**

COS-7 cells (Riken RCB0539) were plated in a concentration of $2 \times 10^6$ cells/10 cm dish, and cultured overnight. Ten micrograms of the

expression vector plasmid constructed as mentioned above (or vector plasmid pcDNA4 HisMax as control) was transfected into COS-7 cells using together with lipofection reagent (trade name: SuperFect, manufactured by Qiagen), and thereafter the transfected cells were cultured for additional 48 hours.

**[0100]**

Using the cells after the culture, overexpressed GS21015 (N-terminal, His tagged) was detected by Western blotting method as follows. Concretely, the cells after the culture were harvested, and thereafter 500 μl of buffer (10 mM HEPES pH 7.5, 1 mM EDTA, 0.34 M sucrose, 1 mM PMSF, 0.1 M DTT) was added thereto to lyse the cells. The cell lysate was centrifuged (15000 rpm for 15 minutes). After supernatant was taken, a 10 μl portion thereof was subjected to SDS-polyacrylamide gel electrophoresis.

**[0101]**

Next, the electrophoresed protein was transferred to a PVDF membrane (trade name: Immobilon-P membrane, manufactured by Millipore) by semi-dry method. Thereafter, the membrane was immersed overnight at 4°C in a solution containing a blocking reagent (Blockace, manufactured by Dainippon Pharmaceutical Co., Ltd.), thereby blocking the membrane. Further, the blocked membrane was reacted with anti-His tag antibody (manufactured by QIAGEN) (1000-fold dilution) at room temperature for 2 hours, and thereafter the reaction product was washed with PBS-Tween (phosphate buffered physiological saline containing 0.1% Tween). Subsequently, the membrane was reacted with peroxidase-labeled anti-mouse IgG antibody (manufactured by Sigma) at room temperature for 2 hours, and thereafter washed. Furthermore, a protein band bound to the anti-His tag antibody in the membrane was detected using a color

developing reagent (ECL System, manufactured by Amersham).

**[0102]**

As a result, a main positive band was recognized around a molecular weight of about 97 kD corresponding to tagged GS21015.

**[0103]**

Example 5 Determination of Phospholipase $A_2$ Activity of GS21015

The phospholipase $A_2$ activity of GS21015 was examined by determining the activity of hydrolyzing a substrate (activity of hydrolyzing an ester bond at 2-position to release arachidonic acid) for the supernatant of the cell lysate collected in Example 4 mentioned above, in which 1-palmitoyl-2-arachidonyl-phosphatidylcholine was used as the substrate, as follows.

**[0104]**

First, 1-palmitoyl-2-[$^{14}$C]arachidonyl-phosphatidylcholine (manufactured by Life Science Products) dried under a nitrogen gas was added to a reaction buffer [10 mM HEPES pH 7.5, 10 mM calcium chloride, 150 mM sodium chloride, 30% glycerol, 1 mg/ml bovine serum albumin (fatty acid-free)] so as to have a concentration of 2 μM. The mixture was stirred, immersed in a bathtub-type ultrasonic washing machine for 30 minutes, and thereafter stirred again. To 250 μl of this substrate solution was added 10 μl of supernatant of cell lysate obtained by transfection of the expression vector plasmid (or as a control, supernatant of the cell lysate into which pcDNA4 HisMax was transfected), each collected in Example 4 mentioned above, to initiate the reaction. After the reaction was carried out while keeping the temperature at 37°C for 0, 15, 30 or 60 minutes, 1.25 ml of a Dole's reagent (2-propanol : heptane : 1 N sulfuric acid = 20:5:1) was added thereto to stop the reaction. Further, 0.1 g of silica gel

for column chromatography (manufactured by Fuji Silysia Chemical Ltd.) was added thereto and stirred, and 0.75 ml of heptane and 0.75 ml of ion-exchanged water were added thereto. The mixture was stirred for 10 seconds or longer. The upper layer (heptane layer) was taken in an amount of 0.5 ml, and mixed with 5 ml of a scintillation solution. Subsequently, the radioactivity was determined with a scintillation counter.

**[0105]**

The results (correlation between the reaction time and the activity) are as shown in Figure 3. It was made clear that GS21015 has a $PLA_2$ activity.

**[0106]**

Example 6 Expression Analysis of GS21015 by *in situ* Hybridization

Expression of GS21015 mRNA in the human skin tissue was analyzed by carrying out *in situ* hybridization using a psoriatic skin tissue and a normal skin tissue from human.

**[0107]**

As the probe for *in situ* hybridization, there was used a cRNA probe obtained as follows. A fragment (nucleotide sequence of the fragment corresponding to a nucleotide sequence of 2522nd to 3236th in the nucleotide sequence shown in SEQ ID NO: 8) of the GS21015 gene (full length cDNA) obtained in Example 3 mentioned above was subcloned into pBluescript vector (manufactured by Stratagene). The resulting plasmid vector was digested with restriction enzymes *Xba*I and *Acc*I and thereafter transcribed with T7 RNA polymerase and T3 RNA polymerase using the resulting product as a template. In addition, the preparation of a skin tissue section and *in situ* hybridization were carried out in accordance with the method described in a literature (Shintaro

Nomura et al., *Saibokogaku Bessatsu (9) "Datsu-aisotopu Jikken Purotokoru (1) DIG Haiburidaizeishon (Cell Technology Supplement (9), De-Isotope Experimental Protocol (1) DIG Hybridization),* 72-82, 1994).

**[0108]**

As a result, it was found that GS21015 mRNA is expressed in the upper layer of psoriatic epidermis. Also, little expression was detected in normal skin tissues. Therefore, there was confirmed increase in expression of GS21015 mRNA in psoriatic skin tissues as compared to normal skin tissues.

**[0109]**

Example 7 Expression Analysis of GS21015 by Northern Blotting Method

Expression of GS21015 mRNA in various human tissues was analyzed by carrying out Northern blotting using commercially available membranes into which mRNAs from various human tissues [GeneHunter (code numbers: MRB-111, MRB-112, MRB-113, MRB-114, MRB-311, and MRB-312), manufactured by Toyobo Co., Ltd.] were blotted.

**[0110]**

As the probe in Northern blotting, there was used a fragment (nucleotide sequence of the cDNA fragment corresponding to 99th to 770th nucleotide sequence of the nucleotide sequence shown in SEQ ID NO: 8) of the GS21015 gene (full length cDNA) obtained in Example 3 mentioned above by labeling with $^{32}P$.

**[0111]**

As a result of Northern blotting, strong expression of GS21015 mRNA was found in fetal skin tissues and adult uterus cervical tissues, and weak expression was found in adult uterus tissues and adult prostate tissues. However,

expression was not found in other tissues. It has been known that $cPLA_2\alpha$, $cPLA_2\beta$, and $cPLA_2\gamma$ are expressed in almost all the tissues (Pickard et al., *The Journal of Biological Chemistry*, **274**, 8823-8831, 1999). In this respect, it was found that the GS21015, is significantly different from the known $cPLA_2$.

**[0112]**

"SEQUENCE FREE TEXT"

Free Text for SEQ ID NOs: 1 to 3 and 5 to 7

<223> Artificially synthesized primer sequence.

[Sequence Listing]

SEQUENCE LISTING

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<130> A00-4783

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Gly Gly Pro Thr Gly His Pro Tyr Gln Gly Glu Ala Ser Thr Cys Trp
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Gln Leu Thr Val Arg Val Leu Glu Ala Arg Asn Leu Arg Trp Ala Asp
25 30 35

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Leu Leu Ser Glu Ala Asp Pro Tyr Val Ile Leu Gln Leu Ser Thr Ala
40 45 50

cct gga atg aag ttt aag acc aag acg ctc acc gac acc agt cat cct 308
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ggc atc tct ggc tct acg tgg aca atg gcc cac ctg tac ggg gac cct 1220
Gly Ile Ser Gly Ser Thr Trp Thr Met Ala His Leu Tyr Gly Asp Pro
360 365 370

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Glu Trp Ser Gln Arg Asp Leu Glu Gly Pro Ile Arg Tyr Ala Arg Glu
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cac ctg gcc aag agc aag ctg gag gtc ttt tcc cca gag cgc ctg gcg 1316
His Leu Ala Lys Ser Lys Leu Glu Val Phe Ser Pro Glu Arg Leu Ala
395 400 405

agc tac cgc cgg gag ctg gag ctg cgg gct gag cag ggc cac ccc acg 1364
Ser Tyr Arg Arg Glu Leu Glu Leu Arg Ala Glu Gln Gly His Pro Thr
410 415 420

acc ttt gtg gac ctg tgg gcg cta gtg ctg gag tcc atg ctg cac ggc 1412

Thr Phe Val Asp Leu Trp Ala Leu Val Leu Glu Ser Met Leu His Gly
425 430 435

cag gtg atg gat cag aag ctg tca gga cag aga gcc gcc ctg gaa cgg 1460
Gln Val Met Asp Gln Lys Leu Ser Gly Gln Arg Ala Ala Leu Glu Arg
440 445 450

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Gly Gln Asn Pro Leu Pro Leu Tyr Leu Ser Leu Asn Val Lys Glu Asn
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Asn Leu Glu Thr Leu Asp Phe Lys Glu Trp Val Glu Phe Ser Pro Tyr
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600 605 610

tac tgt agc cac aaa gac ttc tcc acc tgg gca gac tac cag ctt gac 1988
Tyr Cys Ser His Lys Asp Phe Ser Thr Trp Ala Asp Tyr Gln Leu Asp
615 620 625 630

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Ser Met Pro Ser Gln Leu Thr Pro Lys Glu Pro Arg Leu Cys Leu Val
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695 700 705 710

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Arg Glu Cys His Leu Phe Ser Asp Pro Ala Cys Pro Glu Ala Pro Ile
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Leu Leu His Phe Pro Leu Val Asn Ala Ser Phe Lys Asp His Ser Ala
730 735 740

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745 750 755

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Thr Ser Gln Gly Ala Ile Leu Gln Ala Leu Arg Thr Ala Leu Lys His
795 800 805

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Arg Thr Leu Glu Ala Arg Pro Pro Arg Ala Gln Thr
810 815

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atgta 3587

<210> 9

<211> 818

<212> PRT

<213> Homo sapiens

<400> 9

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20 25 30

Asn Leu Arg Trp Ala Asp Leu Leu Ser Glu Ala Asp Pro Tyr Val Ile
35 40 45

Leu Gln Leu Ser Thr Ala Pro Gly Met Lys Phe Lys Thr Lys Thr Leu
50 55 60

Thr Asp Thr Ser His Pro Val Trp Asn Glu Ala Phe Arg Phe Leu Ile
65 70 75 80

Gln Ser Gln Val Lys Asn Val Leu Glu Leu Ser Ile Tyr Asp Glu Asp
85 90 95

Ser Val Thr Glu Asp Asp Ile Cys Phe Lys Val Leu Tyr Asp Ile Ser
100 105 110

Glu Val Leu Pro Gly Lys Leu Leu Arg Lys Thr Phe Ser Gln Ser Pro
115 120 125

Gln Gly Glu Glu Glu Leu Asp Val Glu Phe Leu Met Glu Glu Thr Ser
130 135 140

Asp Arg Pro Glu Asn Leu Ile Thr Asn Lys Val Ile Val Ala Arg Glu
145 150 155 160

Leu Ser Cys Leu Asp Val His Leu Asp Ser Thr Gly Ser Thr Ala Val
165 170 175

Val Ala Asp Gln Asp Lys Leu Glu Leu Glu Leu Val Leu Lys Gly Ser
180 185 190

Tyr Glu Asp Thr Gln Thr Ser Phe Leu Gly Thr Ala Ser Ala Phe Arg
195 200 205

Phe His Tyr Met Ala Ala Leu Glu Thr Glu Leu Ser Gly Arg Leu Arg
210 215 220

Ser Ser Arg Ser Asn Gly Trp Asn Gly Asp Asn Ser Ala Gly Tyr Leu
225 230 235 240

Thr Val Pro Leu Arg Pro Leu Thr Ile Gly Lys Glu Val Thr Met Asp
245 250 255

Val Pro Ala Pro Asn Ala Pro Gly Val Arg Leu Gln Leu Lys Ala Glu
260 265 270

Gly Cys Pro Glu Glu Leu Ala Val His Leu Gly Phe Asn Leu Cys Ala
275 280 285

Glu Glu Gln Ala Phe Leu Ser Arg Arg Lys Gln Val Val Ala Lys Ala
290 295 300

Leu Lys Gln Ala Leu Gln Leu Asp Arg Asp Leu Gln Glu Asp Glu Val

305 310 315 320

Pro Val Val Gly Ile Met Ala Thr Gly Gly Gly Ala Arg Ala Met Thr
325 330 335

Ser Leu Tyr Gly His Leu Leu Ala Leu Gln Lys Leu Gly Leu Leu Asp
340 345 350

Cys Val Thr Tyr Phe Ser Gly Ile Ser Gly Ser Thr Trp Thr Met Ala
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His Leu Tyr Gly Asp Pro Glu Trp Ser Gln Arg Asp Leu Glu Gly Pro
370 375 380

Ile Arg Tyr Ala Arg Glu His Leu Ala Lys Ser Lys Leu Glu Val Phe
385 390 395 400

Ser Pro Glu Arg Leu Ala Ser Tyr Arg Arg Glu Leu Glu Leu Arg Ala
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Glu Gln Gly His Pro Thr Thr Phe Val Asp Leu Trp Ala Leu Val Leu
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Glu Ser Met Leu His Gly Gln Val Met Asp Gln Lys Leu Ser Gly Gln
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Arg Ala Ala Leu Glu Arg Gly Gln Asn Pro Leu Pro Leu Tyr Leu Ser
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Leu Asn Val Lys Glu Asn Asn Leu Glu Thr Leu Asp Phe Lys Glu Trp
465 470 475 480

Val Glu Phe Ser Pro Tyr Glu Val Gly Phe Leu Lys Tyr Gly Ala Phe
485 490 495

Val Pro Pro Glu Leu Phe Gly Ser Glu Phe Phe Met Gly Arg Leu Met
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Arg Arg Ile Pro Glu Pro Arg Ile Cys Phe Leu Glu Ala Ile Trp Ser
515 520 525

Asn Ile Phe Ser Leu Asn Leu Leu Asp Ala Trp Tyr Asp Leu Thr Ser
530 535 540

Ser Gly Glu Ser Trp Lys Gln His Ile Lys Asp Lys Thr Arg Ser Leu
545 550 555 560

Glu Lys Glu Pro Leu Thr Thr Ser Gly Thr Ser Ser Arg Leu Glu Ala
565 570 575

Ser Trp Leu Gln Pro Gly Thr Ala Leu Ala Gln Ala Phe Lys Gly Phe
580 585 590

Leu Thr Gly Arg Pro Leu His Gln Arg Ser Pro Asn Phe Leu Gln Gly
595 600 605

Leu Gln Leu His Gln Asp Tyr Cys Ser His Lys Asp Phe Ser Thr Trp
610 615 620

Ala Asp Tyr Gln Leu Asp Ser Met Pro Ser Gln Leu Thr Pro Lys Glu
625 630 635 640

Pro Arg Leu Cys Leu Val Asp Ala Ala Tyr Phe Ile Asn Thr Ser Ser
645 650 655

Pro Ser Met Phe Arg Pro Gly Arg Arg Leu Asp Leu Ile Leu Ser Phe
660 665 670

Asp Tyr Ser Leu Ser Ala Pro Phe Glu Ala Leu Gln Gln Thr Glu Leu
675 680 685

Tyr Cys Arg Ala Arg Gly Leu Pro Phe Pro Arg Val Glu Pro Ser Pro
690 695 700

Gln Asp Gln His Gln Pro Arg Glu Cys His Leu Phe Ser Asp Pro Ala

705 710 715 720

Cys Pro Glu Ala Pro Ile Leu Leu His Phe Pro Leu Val Asn Ala Ser
725 730 735

Phe Lys Asp His Ser Ala Pro Gly Val Gln Arg Ser Pro Ala Glu Leu
740 745 750

Gln Gly Gly Gln Val Asp Leu Thr Gly Ala Thr Cys Pro Tyr Thr Leu
755 760 765

Ser Asn Met Thr Tyr Lys Glu Glu Asp Phe Glu Arg Leu Leu Arg Leu
770 775 780

Ser Asp Tyr Asn Val Gln Thr Ser Gln Gly Ala Ile Leu Gln Ala Leu
785 790 795 800

Arg Thr Ala Leu Lys His Arg Thr Leu Glu Ala Arg Pro Pro Arg Ala
805 810 815

Gln Thr

[Brief Description of the Drawings]

[Figure 1] A diagram showing results of analysis of an expression level

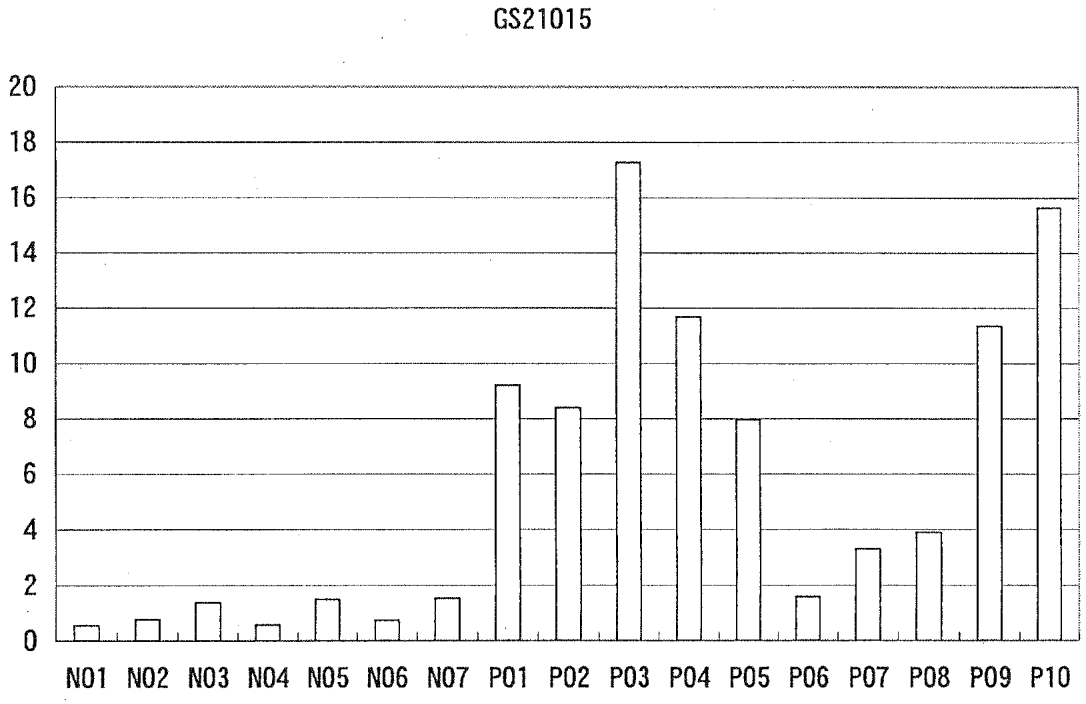
of a GS21015 gene in skin tissue samples from a normal individual and a patient with psoriasis by iAFLP method; in the figure, "N01", "N02", "N03", ... and "N07" indicate a tissue sample of a normal individual, and "P01", "P02", "P03", ... and "P10" indicate a tissue sample of a patient with psoriasis.

[Figure 2] A diagram showing homology obtained by comparing an amino acid sequence of GS21015, and amino acid sequences of $cPLA_2\alpha$, $cPLA_2\beta$ and $cPLA_2\gamma$ which have conventionally been known as molecular species of $cPLA_2$.

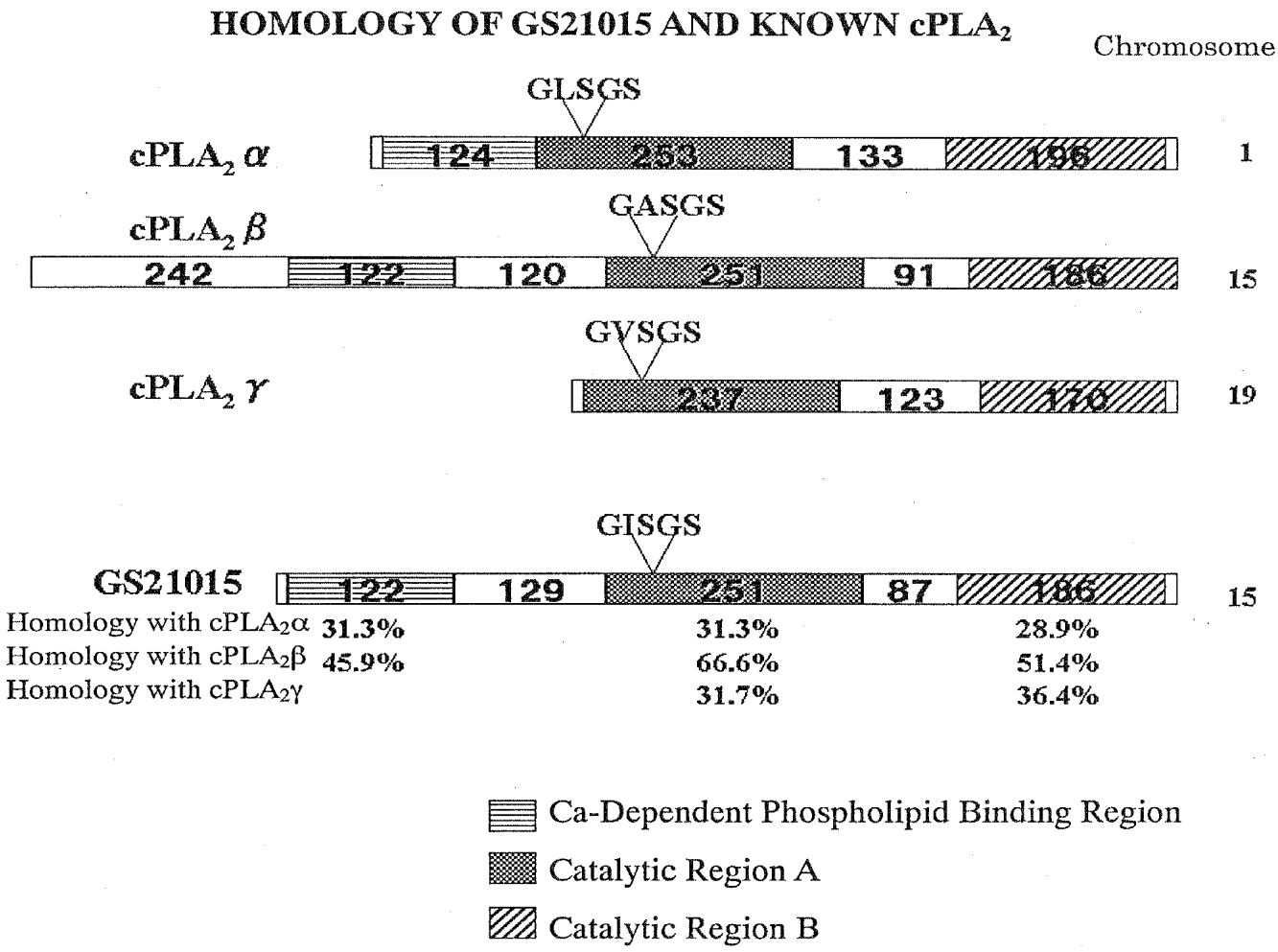
[Figure 3] A diagram showing correlation between phospholipase $A_2$ activity of GS21015 (activity of hydrolyzing an ester bond of 1-palmitoyl-2-arachidonyl-phosphatidylcholine at 2-position, to release arachidonic acid) and a reaction time.

[Document] Drawings

[Figure 1]



[Figure 2]


HOMOLOGY OF GS21015 AND KNOWN cPLA2
Chromosome
GLSGS
cPLA2 α
124
253
133
196
1
GASGS
cPLA2 β
242
122
120
251
91
186
15
GVSGS
cPLA2 γ
237
123
170
19
GISGS
GS21015
122
129
251
87
186
15
Homology with cPLA2α 31.3% 31.3% 28.9%
Homology with cPLA2β 45.9% 66.6% 51.4%
Homology with cPLA2γ 31.7% 36.4%
Ca-Dependent Phospholipid Binding Region
Catalytic Region A
Catalytic Region B

[Figure 3]

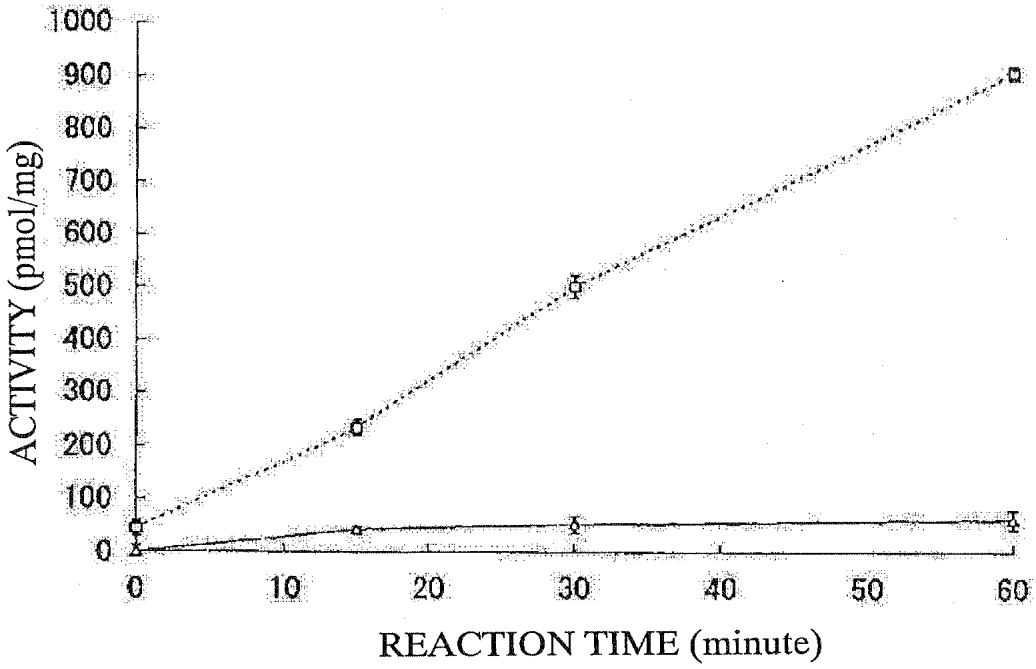



**Figure 3 PHOSPHOLIPASE $A_2$ ACTIVITY OF GS21015**

[Document] Abstract

[Abstract]

[Problems] To provide a novel type of phospholipase $A_2$ ($PLA_2$) and a gene thereof associated with psoriasis, a novel method for characterizing, identifying or screening a phospholipase $A_2$ inhibitor, and a novel method for diagnosis or examination of psoriasis.

[Solving Means]

A protein selected from the following (a), (b) and (c):

(a) a protein consisting of the amino acid sequence shown in SEQ ID NO: 9;

(b) a protein consisting of an amino acid sequence in which one or more amino acids in the amino acid sequence shown in SEQ ID NO: 9 are deleted, substituted or added, wherein the protein possesses a phospholipase $A_2$ activity; and

(c) a protein encoded by a DNA capable of hybridizing with a DNA consisting of a nucleotide sequence complementary to the nucleotide sequence shown in SEQ ID NO: 8 under stringent conditions, wherein the protein possesses a phospholipase $A_2$ activity; and

a gene encoding the protein; and the like.

[Selected Drawings] None

# ACCREDITED AND ADDED INFORMATION

| | |
|---|---|
| Patent Application Number | Japanese Patent Application No. 2002-008435 |
| Reception Number | 50200052155 |
| Document | Petition for Patent Application |
| Examiner in Charge | Head of Fifth Person in Charge 0094 |
| Date Prepared | January 18, 2002 |

< Accredited Information/Added Information >

| | |
|---|---|
| [Filing Date] | January 17, 2002 |

# BACKGROUND INFORMATION OF APPLICANT

| | | |
|---|---|---|
| Identification Number | | [000002956] |
| 1. | Date of Conversion | September 20, 1990 |
| | [Reason for Conversion] | New Registration |
| | Address | 2-10, Dosho-machi 3-chome, Chuo-ku, Osaka-shi, Osaka |
| | Name | TANABE SEIYAKU CO., LTD. |